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Microbac Protocol

PRE-SATURATED OR IMPREGNATED TOWELETTE VIRUCIDAL EFFICACY TEST -

Murine Norovirus

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
STERIS CORPORATION
7405 Page Avenue
St. Louis, MO 63133-1032

October 23, 2017

Microbac Protocol: 429.1a.10.23.17

Microbac Project: 429-399

OBJECTIVE:

This test is designed to determine the disinfectant effectiveness for impregnated or pre-saturated towelettes, single use, to be registered with the United States Environmental Protection Agency (EPA). The test follows the "Germicidal Spray Products as Disinfectants" test as described in the Official Methods of Analysis, Nineteenth Edition, 2013, AOAC, the procedure outlined in the ASTM International test method designated E1053-11, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces" and the EPA Notice of Efficacy Requirements for Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection. This test also meets the EPA OCSPP 810.2000 and 810.2200 Product Performance Test Guidelines, and Health Canada "Guidance Document – Safety and Efficacy Requirements for Hard Surface Disinfectant Drugs" (January 2014).

TESTING CONDITIONS:

Virus will be dried on sterile glass Petri dishes at ambient temperature. One test substance (towelette), two lots, will be tested at one exposure (contact) time at one replicate (N=1). The test substance will be used to treat the dried virus inoculum according to the label claims or Sponsor specification if provided. One carrier, at 10-square inches surface area, will be tested for each run. After a defined exposure period, the test substance-virus mixture will be scraped from the surface, collected, neutralized and assayed for the presence of infectious virions.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page). The chemical characterization will be appended to the final report. As per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined for each batch and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each batch.

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures Microbac testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused test substances after completion of the test for one year, and will only discard them with client permission in a manner that meets the approval of the safety officer.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge virus (requested by the sponsor of the study): Murine Norovirus, strain: MNV-G, Yale University

    - Note: the virus inoculum will contain 5% serum organic load.

2. Host cell line: RAW 264.7, ATCC TIB-71

3. Laboratory equipment and supplies.

4. Media and reagents:

    Media and reagents relevant to the virus-host system and test substance being tested will be documented in the first project sheet and data pack.

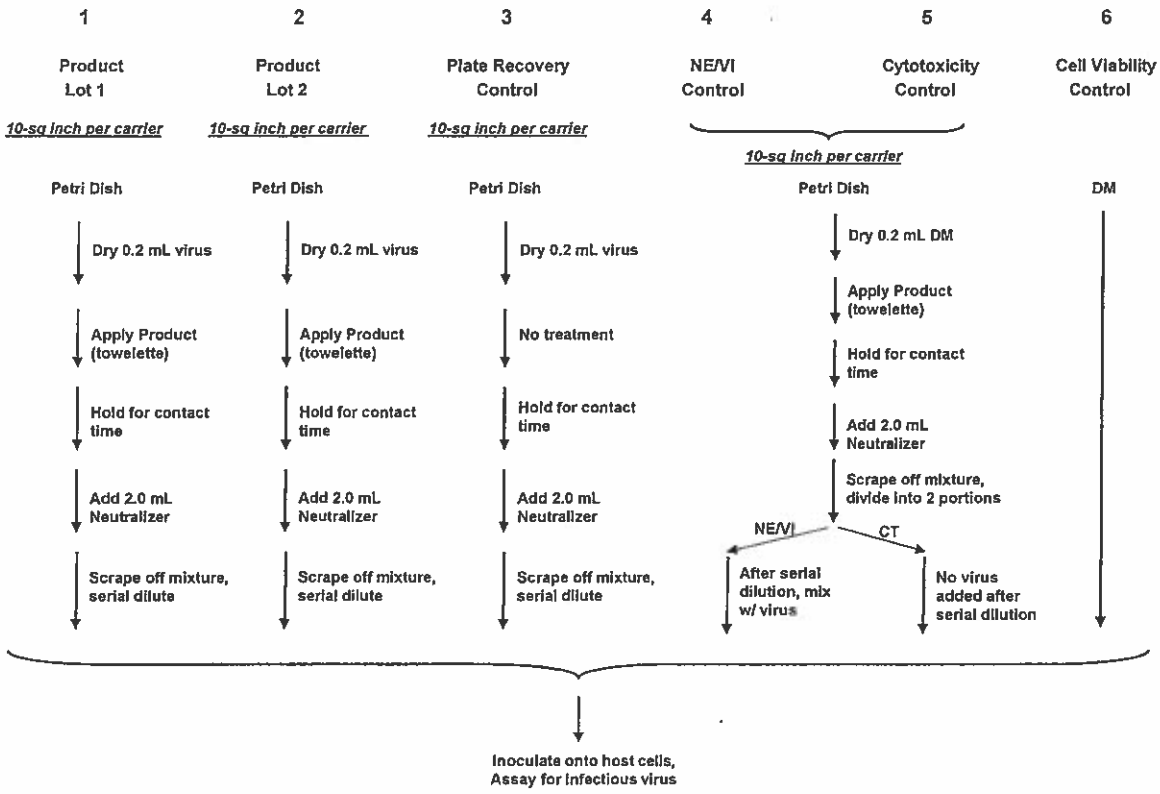
TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test substance and/or project number.

EXPERIMENTAL DESIGN:

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at Microbac. SOPs and Logs are referred to in the raw data and are required as part of GLP regulations. The study flow diagram is shown in Figure 1, with details described in the following sections.

FIGURE 1



DM: Dilution Medium
CT: Cytotoxicity Control
NE/VI: Neutralizer Effectiveness/Viral Interference control

Note: One test substance (towelette), two lots, will be tested at one exposure (contact) time and one replicate (N=1). The NE/VI and CT controls will be performed at one replicate per lot.

A. Inoculum preparation:

Viral stocks are originally purchased from reputable sources that identify them by scientifically accepted methods and may have been propagated at Microbac. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test (fresh stock cultures may be used at the discretion of the Study Director). The stock virus will have an organic load of at least 5% serum unless otherwise specified by the Sponsor and pre-approved by Microbac.

Note: a level of approximately 4.0 – 6.0 Log_{10} virus challenge (as indicated by the plate recovery control load) when there is no cytotoxicity, or approximately 3.0 – 5.0 Log_{10} beyond the level of cytotoxicity, if present, should be achieved whenever possible.

B. Carrier preparation:

One carrier with 10-square inches of surface area (i.e. equivalent to the surface area of ten of one-square inch carriers) will be prepared for each lot of the test substance. For each carrier, an aliquot of 0.2 mL virus will be added, and spread with a cell scraper, over the entire area of approximately 10 square inches (for example, 3.33 inches long x 3 inches wide, or equivalent) that has been marked on the underside of pre-sterilized glass Petri dishes. Carriers treated with virus will be dried at room temperature and both the drying time and temperature will be recorded.

One carrier will be prepared for each lot of the test substance using virus. One carrier will be prepared for the plate recovery control using virus. Additionally, one carrier will be prepared for each lot of the neutralizer effectiveness/viral interference and cytotoxicity controls using medium in lieu of virus as the inoculum.

C. Test substance preparation:

Note: Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study.

The test substances will be prepared exactly according to the sponsor's directions (if provided).

D. Test:

Note: The temperature and humidity level of the laboratory during the test phase will be monitored and reported.

One test substance (towelette), two lots, will be tested at one exposure (contact) time and one replicate (N=1). One piece of towelette will be used to treat each 10-square inch carrier based on the following:

Immediately before testing, pressure will be applied to the pouch containing the wipes and the liquid will rupture the bladder to saturate the wipes. The test substance will be allowed to equilibrate to ambient room temperature for at least 10 minutes before testing.

Each carrier will be wiped using one piece of towelette, following the below procedure as requested by the sponsor:

Fold the towelette lengthwise in half two times. The far end will be folded up once and the folding will be repeated in the same direction so that an additional 4 folds of the same size are made resulting in a total of 5 folds. The outside edges will then be pulled upward to form a "U" shape when wiping. Handling of the towelette should be minimal and no liquid should be expressed during folding. If a substantial amount of liquid is expressed during folding, discard the towelette and a new one should be used.

Each carrier will be wiped back and forth two times lengthwise with the towelette for a total of four motions across the entire inoculum. The carrier may be treated in sections to ensure sufficient contact of the entire contaminated area. If the carrier is treated in sections, a new, unused area of the folded towelette will be exposed for each section. The sections should be treated in such a way that overlapping is minimal.

After wiping, a stopwatch will immediately be started. The carrier will be held for the exposure (contact) time as specified by the Sponsor. Upon completion of the contact time, the virus and test substance test mixture will be neutralized with 2.0 mL of neutralizer solution and the mixture will be scraped off from the surface of the dish with a cell scraper. This post-neutralized sample (PNS) is considered "10^{-1}".

Using dilution test tubes and appropriate pipette, an aliquot of the PNS will be used for making serial 10-fold dilutions in DM (for example, 0.5 mL sample + 4.5 mL DM). Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section.

Depending upon the test substance, the PNS may be further quenched by a gel-filtration column (e.g., Sephacryl column). If columns are used, the PNS will be passed through pre-spun Sephacryl columns. The columns will be spun for 3 minutes at 1000 rpm. The pass-through samples will be collected and serially tenfold diluted in dilution medium (DM). Selected dilutions will be inoculated onto the host cells as described in the "Infectivity Assay" section.

E. Controls:

1. Plate recovery control (PRC):

This control will be performed in one replicate. The virus inoculum will be spread over the surface of a sterile glass Petri dish and left to dry at ambient temperature. The carrier will be held for the same contact time as for the test substance-carriers, but without any towelette treatment. Post contact time this sample will be applied with 2.0 mL of the neutralizer and processed as the test. Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in Section F "Infectivity Assay". This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

The result from this control will be compared with the test results to confirm recovery of at least 4.0-log of infectious virus in this control following drying and neutralization. Its titer will be used to compare with the titer of the test results to determine the log reduction of the test substance(s).

2. Neutralizer effectiveness/Viral interference control (NE/VI):

This control will determine if residual active ingredient is present after neutralization and if the neutralized test substance interferes with the virus infection system. This control will be performed for both lots of the test substances at one replicate per lot.

The test substance will be processed exactly as the test procedure but in lieu of virus inoculum, dried medium will be exposed to the test substance and assayed as previously described. Post-treatment and neutralization, the neutralized DM/test substance mixture will be divided into two portions, one for cytotoxicity control and the other for neutralizer effectiveness/viral interference control, and processed as the test.

The neutralizer effectiveness/viral interference control sample will be diluted as follows: using dilution test tubes and appropriate pipette, an aliquot of the PNS will be used for making serial 10-fold dilutions in DM (for example, 0.5 mL sample + 4.5 mL DM). Following serial dilution, 0.1 mL of a low titered virus, containing no more than approximately 5,000 infectious units of virus, will be added to 4.5 mL of each dilution and held for a period of no shorter than the contact time. Then these samples will be used to inoculate host cells as described for the test procedure.

Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in Section F, "Infectivity Assay".

3. Cytotoxicity control (CT):

This control will be performed for both lots of the test substance at one replicate per lot.

The cytotoxicity sample, acquired from the neutralizer effectiveness/viral interference control run, will be diluted to simulate testing, and have no virus added. The sample will be diluted as follows: using dilution test tubes and appropriate pipette, 0.5mL aliquot of the PNS will be used for making serial 10-fold dilutions in DM (0.5 mL sample + 4.5 mL DM). Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in Section F, "Infectivity Assay". These effects are distinct from virus-induced cytopathic effects, which will be evident in the plate recovery control cultures.

4. Cell viability control:

This control will be performed at one replicate. It will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will

confirm the sterility of the DM employed throughout the assay period. At least four wells of cells will receive only DM and will be incubated and processed with both test and other controls. This will serve as the negative control.

5. Virus Stock Titer control (VST):

This control will be performed at one replicate. An aliquot of the virus used in the study will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

6. Column titer control (to be performed only if Sephacryl columns are used):

This control will be performed only if Sephacryl columns are used. It is performed to determine any effects of Sephacryl columns on infectious virus titer while passing through the columns. The sample for this control will be acquired from a portion of the PRC prior to passing through the columns, will be used to make direct ten-fold serial dilutions in DM, and processed in the same manner as the rest of the test and controls.

F. Infectivity assay:

The residual infectious virus in test and control samples will be detected by viral-induced cytopathic effect (CPE).

Following aspiration of the growth media, selected dilutions of the neutralized inoculum/disinfectant test substance mixture will be added to cultured host cells (at least four wells per dilution, per reaction mixture) and incubated at 36±2°C with 5±3% CO_2 for total 4 – 7 days. The cell culture may be washed twice with phosphate buffered saline prior to the inoculation. The inoculated culture will be observed and refed with fresh media as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will then be examined microscopically for presence of infectious virions. The resulting virus-specific cytopathic effects and test substance-specific cytotoxic effects will be scored by examining both test and controls. These observations will be recorded.

G. Calculation:

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber. The test results will be reported as the reduction of the virus titer due to treatment with test substance expressed as log_{10}. No statistical analysis will be used for this test.

The Virus Load will be calculated in the following manner:
Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume per sample (mL)]

The Log_{10} Reduction Factor (LRF) will be calculated in the following manner:
Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$) – Output viral load (Log_{10} $TCID_{50}$)

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The infectious virus recovered from the PRC control must be ≥ 4.0-log_{10}.
- Viral-induced cytopathic effect (if any) must be distinguishable from test substance-induced cytotoxic effects.
- Virus must be recovered from the neutralizer effectiveness/viral interference controls (not exhibiting cytotoxicity).
- Cell viability control and cytotoxicity control must be negative for infectivity.

PRODUCT EVALUATION CRITERIA:

According to the US Environmental Protection Agency, the test substance passes the test if there is complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at Microbac, 105 Carpenter Drive, Sterling, VA 20164.

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (GLP studies only):

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices (GLP) regulations, 40 CFR 160 (note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study unless otherwise stated).

The Quality Assurance Unit of Microbac will inspect the conduct of the study for GLP compliance. The dates of the inspections and the dates that findings are reported to the study management and study director will be included in the final report.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report. The sponsor will sign the project sheet(s) to acknowledge the change in the protocol.

REPORT FORMAT:

The report will contain all items required by EPA 810.2200 and be in compliance with EPA PR Notice 2011-3 (replaced PRN 86-5). Microbac employs a standard report format for each test design. Each final report will provide at least the following information:

- Sponsor identification
- Test substance identification
- Type of assay and project number
- Study start and end time (clock time)
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report will be sent to the Sponsor. A draft report will be provided to Sponsor for review prior to finalization of the report

All raw data, protocol, protocol modifications, test substance records, copy of final report, and correspondence between Microbac and the sponsor will be stored in the archives at Microbac, 105 Carpenter Drive, Sterling, VA 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test substance; challenge virus and host cell line monolayers used and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study:

A. Name and address: STERIS CORPORATION
7405 Page Avenue
St. Louis, MO 63133-1032

B. Test substance information:

Test substance name	Berkshire Wipes (EXP16042)	
Batch (Lot) No.	PFR2566A	PFR2566B
Active ingredient(s)	PAA, H_2O_2	PAA, H_2O_2
Level of Active Ingredient(s)	☒ Lower Certified Limit (LCL) ☐ Nominal	☒ Lower Certified Limit (LCL) ☐ Nominal
Manufacture Date	12/28/16	12/28/16
Expiration Date	12/28/17	12/28/17

C. Test conditions:

Contact time	☒ 9.5min
Contact temperature	☒ Ambient (20±1C)
Dilution	☒ Not applicable (Towelette)
Organic load in inoculum	☒ 5% serum
Wiping Instruction	☒ Refer to protocol procedures

D. Precautions/storage conditions and chemical characterization:
MSDS and/or Certificate of Analyses provided: ☒ Yes ☐ No

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MISCELLANEOUS INFORMATION (Continued):

REPORT HANDLING:

The sponsor intends to submit this information to: ☒EPA

STUDY CONDUCT: ☒GLP

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 10/27/17

Printed Name: DANIKLEIN

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____ Date: 11/1/17

Study Director Printed Name: Cory Chiossone